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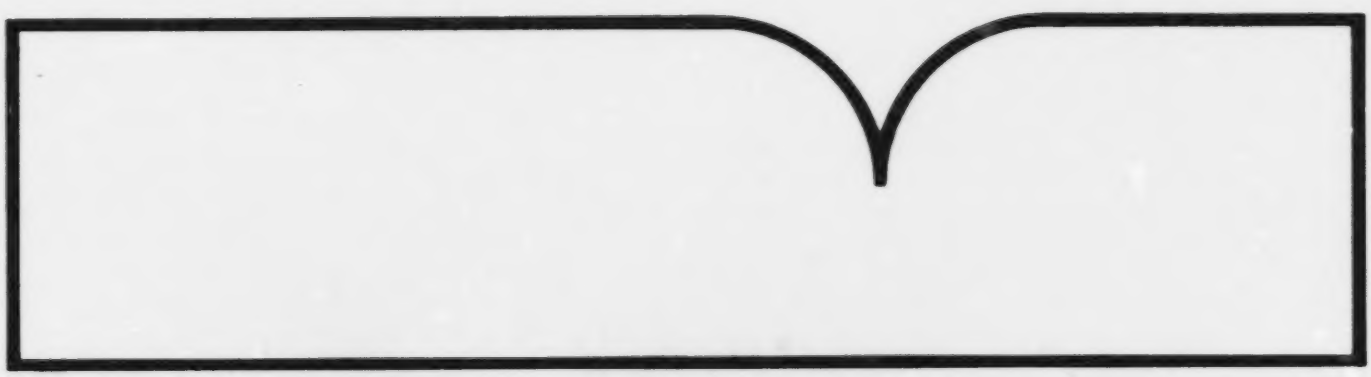

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16. ABSTRACT

In order to examine the response of stream phytoplankton communities to acidification, three artificial streams along the Mississippi River were sampled at biweekly intervals. This study took place at Monticello, Minnesota, during late spring--early summer, 1979. One stream served as a control with an ambient pH of 8.1, and two streams were maintained at pH 6.3 and 5.3 by the addition of sulfuric acid. The streams provided a unique replicate system whereby physical and chemical parameters could be controlled and continually monitored in a field situation. The phytoplankton samples were filtered onto membrane filters and the constituent phytoplankton species were enumerated. The diversity of phytoplankton was similar throughout all three pH regimes. However, phytoplankton community similarity decreased over the course of the six week experimental period. Biomass, measured by in vivo chlorophyll fluorescence and as the density of the algal cells, showed a similar pattern. The pattern of algal community development differed across the pH treatments. The phytoplankton at pH 6.3 and 8.1 attained their maximum biomass during the first month of sampling (June). There is a lag in the population maxima of phytoplankton at pH 5.3, possibly due to a slower division rate caused by a less than ideal pH environment. Species composition was nearly identical across the pH range, dominated by diatoms in each stream. The most extreme pH value, pH 5.3, seemed to be a sublethal value for the diatoms existing there.

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Abstract

In order to examine the response of stream phytoplankton communities to acidification, three artificial streams along the Mississippi River were sampled at biweekly intervals. This study took place at Monticello, Minnesota, during late spring—early summer, 1979. One stream served as a control with an ambient pH of 8.1, and two streams were maintained at pH 6.3 and 5.3 by the addition of sulfuric acid. The streams provided an unique replicate system whereby physical and chemical parameters could be controlled and continually monitored in a field situation. The phytoplankton samples were filtered onto membrane filters and the constituent phytoplankton species were enumerated. The diversity of phytoplankton was similar throughout all three pH regimes. However, phytoplankton community similarity decreased over the course of the six week experimental period. Biomass, measured by *in vivo* chlorophyll fluorescence and as the density of the algal cells, showed a similar pattern. The pattern of algal community development differed across the pH treatments. The phytoplankton at pH 6.3 and 8.1 attained their maximum biomass during the first month of sampling (June). There is a lag in the population maxima of phytoplankton at pH 5.3, possibly due to a slower division rate caused by a less than ideal pH environment. Species composition was nearly identical across the pH range, dominated by diatoms in each stream. The most extreme pH value, pH 5.3, seemed to be a sublethal value for the diatoms existing there.

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Literature Review

Research concerning the effects of increasing hydrogen ion concentration on freshwater organisms has become more prevalent recently. This interest has been spurred by knowledge that fresh water in some areas of the world is especially susceptible to inputs of acid precipitation, resulting in the lowering of pH in these waters. This susceptibility occurs in watersheds which have a poor ability to neutralize acid precipitation due to heavily leached, non-calcareous soils derived from hard crystalline rocks (Gorham 1976). Bodies of fresh water in such areas have a poor buffering capacity and the incoming acids from precipitation have a drastic effect on the water's pH.

On an annual basis, rain and snow over large regions of the world are from 5 to 30 times more acid than the lowest pH value expected (pH 5.6) for unpolluted atmospheres. The rain from individual storms can be from several thousand to several hundred times more acid than expected (Likens et al. 1979). Pure water in equilibrium with atmospheric CO_2 would have a pH of 5.6. Added to this primary source of acidity are strong mineral acids, predominantly sulfuric acid (H_2SO_4) (Gorham 1976). The origin of H_2SO_4 is the oxidation of sulfur in fossil fuels. Also, natural biogenic emissions of sulfur produce acid precipitation, but presumably these sources have been in balance with natural sources of neutralizing bases (Gorham 1976). Oxides of nitrogen (NO and NO_2) are also important sources of acid precipitation. Hydrochloric acid (HCl) is an important source of acidity in some areas, as well. In 1977, sulfur and nitrogen oxides together contributed 26% (50.4 million metric tons) to the total air pollution in the United States (Schaefer 1979).

Another source of acid to fresh water is bituminous coal mine drainage. This acid is formed by the oxidation of sulfur occurring in exposed coal or in rocks adjacent to exposed coal seams. Iron sulfides bound as pyrites, marcasites, and sulfates (in much smaller quantities) are exposed to the oxidizing action of the air, water, and sulfur-oxidizing bacteria such as *Thiobacillus thiooxidans*. The resultant sulfuric acid and ferrous iron drastically increases the acidity of streams receiving acid mine drainage. The annual acid discharge from bituminous coal mines of western Pennsylvania is equivalent to one million tons of sulfuric acid. Downstream from the pollution effluent, the acid ferrous solution is diluted and neutralized and the pH rises. Ferrous iron is slowly oxidized to Fe^{+3} which hydrolyzes to ferric hydroxide: $Fe(OH)_3$. This precipitate covers the stream bottoms with a yellow-brown slime inhibiting the growth of benthic algae and creating a sterile zone in the stream (Parsons 1957; Koryak et al. 1972).

Both planktonic algae and benthic forms are affected by the level of acidity present in fresh water. Transparency of lakewater is enhanced by low pH. Kwaitkowski and Roff (1976) found secchi disc readings to be highly correlated with pH. Turbidity is reduced by less biomass of plankton at lower pH, and also, colloidal particles may become flocculated which increases water clarity. Almer et al. (1974) reported very clear lakes resulting from decreased algae content and the precipitation of humic substances under greatly acidic conditions.

Acidification, and consequently oligotrophication, of lakes is accelerated by retarding the rate of nutrient supply to the primary producers. Bacterial productivity declines with lowering of pH and an

accumulation of coarse detritus in the hypolimnion covers the mineralized sediments, preventing exchange of nutrients and other ions between sediments and the overlying water. Fungi thrive better in acid solutions than bacteria. Fungal hyphae may produce a dense felt on lake bottoms replacing the bacteria as the major decomposers. Fungal decomposition is less efficient than bacterial decomposition resulting in slower nutrient recycling. *Sphagnum*, a moss found in acidic fresh water, has a strong ion exchange capacity and binds ions from the surrounding water, withdrawing these important nutrients from use by other organisms (Grahn et al. 1974). In acid streams, inhibition of bacterial growth (and other organisms' growth) destroys a stream's natural self-purification process. Thus, allochthonous organic matter accumulates in acidic portions of streams, and will not decompose until it reaches a neutralized reach of the stream (Koryak et al. 1972).

Primary productivity is decreased in more acid conditions. Kwaitkowski and Roff (1976) found production (measured in milligrams $C \cdot meter^{-3} \cdot hour^{-1}$) was reduced in lakes below pH 5.5. However, an increase in the depth of the euphotic zone accompanying more acidic conditions kept primary productivity (measured in milligrams $C \cdot meter^{-2} \cdot hour^{-1}$) at high levels down to pH 4.4, below which it was drastically reduced. Oxygen depletion occurred in more neutral lakes, reflecting higher production in the euphotic zone, and thus more decomposition of algal biomass. Johnson et al. (1970) found primary productivity per unit volume generally greater in an unaffected lake than in acid-contaminated lakes. Patrick et al. (1968) found that a pH of 5.2—5.4 affected diatom productivity by slowing the division rate, resulting in lower standing

crops. Furthermore, this effect of acid was more pronounced when water temperature was lower.

Some authors have not found a reduction in algal biomass with increasingly acid conditions. Accumulation of benthic algae in three streams, similar except for pH, was compared by Leivestad et al. (1976). Chlorophyll estimates of algal standing crop were significantly higher at pH 4 than at pH 6 and usually higher than at the natural pH (which ranged from 4.3 to 5.5). These results were attributed to the success of two acid-tolerant filamentous algae at pH 4. Yan et al. (1977) raised the pH of a Canadian Shield lake experimentally from pH 5.7 to 6.7. There was no significant change in biomass. Yan (1979) found that an acidified lake (pH 4.2) had no biomass reduction, but an atypical structure of phytoplankton developed, compared to uncontaminated lakes. Phytoplankton community biomass was more highly correlated with phosphorus concentration than with H^+ ion concentration. Yan and Stokes (1978) stated that biomass should not be used as an index of acidification since it is only a sensitive enough measurement under conditions of extreme acidification. They suggested that measurements of community structure are more sensitive indicators of acidification.

Diversity of algal taxa is one such measurement of community structure. Patrick et al. (1968) measured species diversity in three experiments with attached diatoms. In two of the tests (performed in the winter and in late spring) species diversity decreased at low pH (5.2), compared to more circumneutral conditions. In the third experiment (during the summer) there was no difference in the diversity of diatoms between pH 5.4 and the control (pH range from 7.4—9.6). Johnson et al. (1970) found a

reduction in phytoplankton diversity in La Cloche Mountain lakes contaminated by acid mine drainage when compared to uncontaminated lakes nearby. Leivestad et al. (1976), in a study of 55 Norwegian lakes found a significant correlation between phytoplankton species number observed versus pH. Kwaitkowski and Roff (1976) found the Chlorophyta[1] (green algae) diversity to be especially low in lakes of low pH (4.05—5.65) compared to more neutral lakes. In experiments with polyethylene cylinders filled with lakewater and with pH altered experimentally, Yan and Stokes (1978) reported a reduced diversity of phytoplankton below a pH of 5.8. The number of phytoplankton taxa was reduced by one-half in a contaminated lake (pH 4.2) compared to uncontaminated conditions in a control lake (Yan 1979).

Another useful indication of acid affecting freshwater algae can be seen in changes of species composition. Johnson et al. (1970) noted that lakes contaminated with acid mine wastes had very simple algal compositions consisting of blooms of the blue-green (Cyanophyta) alga *Plectonema notatum*. Uncontaminated, but otherwise similar, lakes nearby had algal assemblages common to lakes of the region consisting of the Chrysophyceae, Cyanophyta, and Bacillariophyceae. In a study of 400 lakes along Sweden's southwest coast, where an influx of acid precipitation from western Europe impinged, Almer et al. (1974) were able to conclude that the species composition of phytoplankton in these lakes was, indeed, related to lake pH. Lakes of pH 4.0 were composed mainly of the Pyrrophyta (*Gymnodinium* spp. and *Peridinium inconspicuum*) and a few chlorophytes (*Ankistrodesmus*

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1. *Taxonomy is after Prescott 1964.*

convolutus, *Oocystis submarina*, and *O. lacustris*). At pH 5.0, lakes commonly had a species composition of diatoms and the blue-greens *Chroococcoccus* and *Merismopedia*. When the pH was greater than 6.0, species composition was diverse and all taxonomic groups were found. The greatest change in algal composition was found between pH 5 and 6. Sediment cores of these lakes revealed that the planktonic diatoms were replaced by other species when acidification of the lake district occurred. Kwaitkowski and Roff (1976) studied lakes similar chemically except for pH (ranging between 4.1 and 7.2) and found the Chlorophyta increased in abundance and diversity with an increase in pH, while the opposite was true for the Cyanophyta. The relative dominance of the Chlorophyta changed from 40—50% at high pH to only 25% in lower pH lakes (below 5.65). Conversely, the relative dominance of the Cyanophyta increased from 30% to 60% when pH was lowered. Yan (1979) found that a Canadian Shield lake of pH 4.2 was predominated by the Pyrrophyta, but stated that the Chrysophyceae would dominate the phytoplankton if the pH was less acidic.

More subtle changes have been observed in samples of benthic algae from streams. Benthic diatom samples from seven locations affected by acid precipitation in Norway were compared between 1949 and 1975. Qualitatively, the diatom flora was similar between the two sampling periods. However, considerable changes had occurred in the proportions of various species, with an increase in the proportion of species which are acidophilous (acid preferring) or acidobiontic (acid requiring) (Leivestad et al. 1976). Patrick et al. (1968) found that experimentally shifting the pH to 5.26 from more circumneutral conditions caused no significant shifts in the kinds of species present. Diatoms accumulating on glass

slides were the same in each stream, but the few species which became dominant at 5.26 were not common in the control condition. It was suggested that the total chemical characteristics of a naturally occurring acid stream are very different from those of a circumneutral stream, and changing only one parameter (pH) would not necessarily cause the development of an acid water flora.

Oftentimes, high levels of acidity in fresh water are accompanied by high concentrations of metals dissolved there. Consequently, it is impossible to separate the effects of these metals from the acid effects. The two together may produce some synergistic effects on algae. In *Hormidium rivulare*, a benthic green alga found in very low pH waters, zinc and copper toxicity has been found to become markedly greater above pH 4.0 (the optimal growth range for *H. rivulare* is pH 3.5 to 4.0) (Hargreaves and Whitton 1976b; Say and Whitton 1977). In a study with five species of algae isolated from extremely low pH habitats, Hargreaves and Whitton (1976a) found growth rates to be reduced at pH 7.0 when iron was present in the growth medium. With iron absent, there was no such reduction of growth and iron's presence at lower pH values did not cause a reduction of growth rate. The authors suggest this may be the result of a direct toxic effect of the metal on the algae, although indirect effects associated with iron precipitation in the water at the higher pH may have been involved.

Just which physiologic features of specific algae determine their tolerance to acidic conditions is a rather unstudied area of phycology. In this, studies involving organisms other than algae can provide some clues. Mosses of the genus *Sphagnum* are very common in acidic freshwater areas. They, in fact, lower the pH of the surrounding medium by exerting

a very high ion exchange capacity. They absorb cations, preferentially those of the highest valence, and release hydrogen ions into the water. This exchanging ability varies with the nature and concentration of the cation, and with the pH of the medium. Correlation has been found between *Sphagnum* cation exchange ability and content of unsterified polyuronic acids in the cell walls (Bell 1959; Clymo 1963). The expulsion of H^+ ions from the cells has also been found to occur in animal cells where H^+ ions and ammonium (NH_4^+) ions are secreted in exchange for sodium (Na^+) uptake, as may occur in stomach lining cells that acidify the external medium (Maetz et al. 1976). Freshwater or anadromous fishes require active salt uptake by specialized epithelial cells, whereby H^+ ions from the body are exchanged for Na^+ ions from the water, and bicarbonate ions are exchanged for chloride ions (Leivestad et al. 1976).

In algae, a cell boundary phenomenon may exist in low pH tolerant species whereby cation exchange or outpumping of H^+ ions by active transport exclude H^+ ions from the cell interior. Additional specializations may include proteins in the cell membrane of tolerant algae that are able to withstand denaturization (which could happen with acid stress) by having very low isoelectric points (Cassin 1974). Brock (1973) suggested that the lower pH limits for the existence of blue-green algae may be due to its procaryotic nature. He hypothesized that eucaryotic cells are potentially more tolerant of acid environments because their chlorophyll is "protected" within membrane-bounded organelles. Cassin (1974) believed it unlikely that hydrogen ions entered cells found at extremely low pH because the chlorophyll in the cells would degrade below pH 5.0 and the cells would no longer be green. However, Lane and Burris (1979) determined that *Chlorella pyrenoidosa*, naturally occurring through

the pH range of 3.0 to 8.0, was able to adjust the pH of the cell's interior depending on the external pH of the medium. The measured internal fluctuation in pH was not as great as the variation in external pH, though.

How algae are able to tolerate high concentrations of heavy metals may provide insight into the tolerance capabilities to hydrogen ions. Sicko-Goad and Stoermer (1979) found that the effects of lead and copper on *Diatoma tenue* var. *elongatum* can be negated by the ability of phosphates in the cells to complex with metals into polyphosphorus bodies. These stored bodies can exist as long as the storage phosphorus is not needed for other essential activities. The toxicity of zinc is ameliorated in *Hormidium rivulare* by the presence of phosphorus, magnesium, and calcium in the external medium. Raising the total hardness and the alkalinity also makes zinc strikingly less toxic (Hargreaves and Whitton 1976b; Say et al. 1977; Say and Whitton 1977). In *Stigeoclonium tenue*, Say and Whitton (1977) reported that lead can be inactivated by its binding onto the cell wall. Large quantities of lead can be found there while the alga is physiologically unaffected by this very toxic metal.

Shifts in the H^+ ion concentration of the water also affects the equilibrium of inorganic carbon dissolved there. Atmospheric CO_2 readily dissolves in water. A reaction of CO_2 with water yields a very dilute solution of carbonic acid. This carbonic acid can dissociate into bicarbonate (HCO_3^-) and carbonate (CO_3^{-2}) forms depending on the water's pH. In the presence of alkaline earth metals, CO_2 becomes HCO_3^- and more CO_2 from the atmosphere can be dissolved. As CO_2 is removed by autotrophs or converted to HCO_3^-, the pH of the water rises. Diel changes in pH can be quite dramatic due to the photosynthetic uptake of CO_2 in fresh water

which has a low buffering capacity (Cole 1975; Talling 1976).

Many researchers have wondered how shifts in pH may affect the ability of algae to take up inorganic carbon for photosynthesis. Schindler (1971; 1972) stated that a shortage of CO_2 in water could practically never occur. Even at very high concentrations of phosphorus and nitrogen, rapid uptake of CO_2 in a low-buffered Canadian Shield lake (with a consequent pH rise to 9.5) failed to cause CO_2 limitation. A pronounced CO_2 gradient between atmosphere and lake epilimnion kept diffusion of CO_2 into the water at a rate high enough to maintain the phytoplankton crop.

Moss (1973a) reported that many oligotrophic algal species which he studied failed to grow above a pH of 8.6, and that eutrophic algae he studied had their best growth rates between pH 8.4 to 9.3 or above. Although hardness of the water was greater at the higher pH, total ionic content was presumably an unlikely factor controlling these observations. He suggested instead that the eutrophic species were able to utilize bicarbonate as a carbon source for photosynthesis while oligotrophic species do not grow above pH 8.6 , since free CO_2 is only available at the lower pH values. It is widely assumed that some algae possess this capability to directly take up HCO_3^- ions while others cannot (Moss 1973a). Goldman (1973) believed that the ability of the procaryotic algae (Cyanophyta) to become dominant at high pH must be explained by something other than the inorganic carbon equilibrium. He suggested that the reason for their success may be explained by enzyme systems that function best at higher pH or nutrients, such as phosphorus, which are more available at the higher pH.

Many algae tolerate extremely acid conditions. Numerous flagellates have been recorded in *Sphagnum* bogs, streams receiving acid mine wastes, and other acid environments: Several species of *Peridinium*, one species of *Spermatozopis*, *Trachelomas volvocina*, *Euglena mutabilis*, *E. viridis*, *Chrysococcous asper*, *Spongemonas uvella*, *Cryptomonas ovata*, and several species of *Chlamydomonas*. *Chlamydomonas acidophila* is extremely resistant to H^+ ion stress, growing better at pH 2.0 than at pH 4.0—5.0. *Euglena mutabilis* is very common in fresh water receiving mine wastes and growth has been documented in water within the pH range 2.1—7.7. Flagellates listed as indicators of high acidity in water supplies include *Chlamydomonas* spp., *Chromulina ovalis*, *Cryptomonas erosa*, *Euglena* spp., *Lepocinclis ovum*, and *Ochromonas* spp. (Lackey 1938; Von Dach 1943; Joseph 1953; Palmer 1962; Bennett 1969; Cassin 1974; and Hargreaves and Whitton 1976a).

Benthic filaments of green algae can be very successful in acid environments, some times clogging streams with dense growths. Commonly occurring types include *Mougeotia* sp., *Hormidium rivulare*, *Stichococcous bacillus*, and *Ulothrix zonata*. Other Chlorophytes common to acid bogs, and generally common to soft water areas, are within the family Desmidiaceae. Among these, *Desmidium* sp. and *Staurastrum* sp. have been recorded in areas receiving acid mine drainage (Lackey 1938; Round 1964; Bennett 1969; Hargreaves and Whitton 1976a; and Leivestad et al. 1976).

The Cyanophyta is generally thought to thrive in neutral to alkaline, eutrophic fresh water (Brock 1973; Moss 1973a). There are, however, exceptions. In studies of several La Cloche Mountain lakes in Ontario (Johnson et al. 1970; Kwaitkowski and Roff 1976), lakes contaminated by

acid input developed a blue-green flora including *Plectonema notatum*, *Aphanocapsa* sp., *Chrococcous dispersus*, *C. limneticus*, and *Oscillatoria* sp.

Many diatoms have long been indicators of acid fresh water in streams. Hustedt (1939) grouped diatoms according to the hydrogen ion concentration in which their occurrence was optimal. In extremely acid streams receiving coal mine wastes, *Navicula viridis*, numerous other Naviculoid species, *Eunotia tenella*, and *Pinnularia braunii* have been found (Lackey 1938; Joseph 1953; Bennett 1969). Besch et al. (1972) studied stream diatoms existing in a low pH environment complemented by high levels of dissolved zinc and copper. Here the dominant species were *Eunotia exigua*, *Achnanthes microcephala*, *A. minutitissima*, *Pinnularia interrupta*, and *Synedra ulna*. Abundant diatoms of the epipelic habit in acid waters include many species of the genera *Eunotia* and *Pinnularia*, *Melosira distans*, *Frustulia rhomboides*, and *Fragilaria virescens* (Round 1964). Leivestad et al. (1976) observed thriving growths of *Tabellaria flocculosa* in a stream of pH 4.0. Patrick et al. (1968) found that the most common diatoms accumulating on artificial substrates at pH 5.26 were *Gomphonema parvalum*, *G. commutatum*, *Navicula pupula*, *Synedra rumpens*, and *Melosira granulata*.

Introduction

Today, more than ever before, atmospheric oxidation of sulfur and nitrogen from anthropogenic sources and their ultimate precipitation is endangering freshwater ecosystems. In especially susceptible areas around the world, the results of acid precipitation and acid coal mine drainage

on freshwater acidity are well documented (Gorham 1976; Likens et al. 1979). The rise in sulfur and nitrogen emissions to the atmosphere accompany the rise in the burning of fossil fuels. Due to wide demand and dependence on increasingly dwindling global oil sources, emissions of nitrogen and sulfur oxides are expected to rise as coal usage increases.

European interest in this problem began after World War II, in the 1940s, when the chemistry of acid rainfall, and later, its effects on freshwater life were studied. Early data from Eastern North America were generally lacking until the 1970s (Gorham 1976). Current knowledge on the effects of high acidity on the freshwater biota includes information on many trophic levels of aquatic ecosystems. Entire populations of fish have been eliminated from heavily acidic lakes (Beamish et al. 1975; Leivestad et al. 1976). The structure of stream zoobenthos and lake zooplankton is also affected. The simplification of food chains results from elimination of non-tolerant species while a general increase in acid-tolerant invertebrates occurs (Koryak et al. 1972; Sprules 1975b).

Algae in fresh water is a primary source of food for aquatic consumers and also plays an important role in reoxygenation (self-purification) of water (Bennett 1969). Changes in phytoplankton communities have accompanied acidification in many studies. The best documented of these changes is a reduction in community diversity which is first evident at pH levels of 5.0—6.0 (Patrick et al. 1968; Johnson et al. 1970; Leivestad et al. 1976; Kwaitkowski and Roff 1976; and Yan and Stokes 1978). Shifts to rather atypical species composition have occurred in phytoplankton (Johnson et al. 1970; Almer et al. 1974) and in benthic algae (Patrick et al. 1968; Leivestad et al. 1976).

Various studies have been concerned with observation of algae present in freshwater bodies of many pH values (Merilainen 1967; Johnson et al. 1970; Besch et al. 1972; Almer et al. 1974), laboratory studies of species tolerance to acidity (Moss 1973a; Cassin 1974; Hargreaves and Whitton 1976b), and studies where pH is extremely low and an algal flora of acid-tolerant species develops (Lackey 1938; Joseph 1953; Bennett 1969). Some investigators have experimentally changed pH to determine its effect on freshwater algae (Patrick et al. 1968; Leivestad et al. 1976; Yan et al. 1977; and Yan and Stokes 1978). The present work studied the phytoplankton in shallow pools of three artificial streams, and compared phytoplankton community structure between three different pH regimes. The system was maintained so practically all physical and chemical parameters were similar during the deliberate addition of acid to these streams.

Materials & Methods

This study was conducted in three outdoor channels at the U. S. Environmental Protection Agency's Monticello Ecological Research Station (MERS) near Monticello, Minnesota. Each stream had 30.5 meter long alternate riffles and pools. The depth and width of the riffles were 10—20 centimeters and 2.4 meters, and of the pools 76—86 centimeters and 3.7 meters. The pools were mud-bottomed while the riffles were constructed with 2—5 centimeter diameter gravel. Experimental water was pumped in from the Mississippi River beginning in April, 1979. Discharge in each channel was measured daily and averaged 761 liters · minute^{-1} over the six week course of the experiment, ranging from 700—847 liters · minute^{-1}.

One channel was maintained with Mississippi River water alone. It was not dosed with acid and thus it served as a control. Here the ambient pH averaged 8.1 and ranged from 7.8—8.6. Two other streams were dosed with technical industrial grade sulfuric acid (see Appendix 1). The acid was stored in a 500-gallon polyethylene tank. The bottom of the tank was connected with CVPC plastic pipe to a variable speed pump. The pump could meter amounts of acid at a rate between 20 and 600 ml/minute. From the pump, the acid was conveyed to the streams with CVPC pipe. The first riffle was deepened and functioned as a mixing compartment. One of these streams was maintained at an average pH of 6.3 (ranging between 6.0—6.6) and the other at an average pH of 5.3 (ranging from 5.0—5.7) (Figure 1). From this point on, the ambient and two experimental streams are referred to as 8, 6, and 5 respectively. Riffles and pools are coded by numbers and letters from the upstream end of each stream. All phytoplankton samples were collected from pools 4, 150 meters below the point of introduction and mixing of acid into the channels.

The upstream riffles and pools of each stream were shaded by A-frame canvas shading modules. The purpose of the shades was to inhibit macrophyte growth in that portion of the streams.

MERS personnel collected information concerning various physical and chemical parameters of the streams. Both morning (5—9 a.m.) and afternoon (3—5 p.m.) temperature readings were taken from mid-depth in pools 3 and 4. Morning and afternoon pH measurements were taken from pools 3, and from riffles c and d. Measurements of dissolved oxygen, specific conductance, hardness, total acidity, and alkalinity were also collected (at mid-depth in pools 3) weekly.

Plankton sampling occurred at two-week intervals from May 30, 1979, to July 11, 1979. Approximately one month of equilibration was allowed under experimental conditions before sampling began. One-liter water samples were taken from mid-depth from pools 4. The samples were stored on ice in the dark during transit to the laboratory (approximately 4 hours) where analyses were conducted. *In vivo* chlorophyll fluorescence (Lorenzen 1966) of 5 ml aliquots from each sample was measured using a Turner Model III Fluorometer equipped with a primary blue filter allowing a maximum transmittion of light at 430 nm and a secondary red filter allowing a maximum transmittion at 650 nm. Dry weights and ash-free dry weights were obtained for all samples. Two 250 ml samples from each sampled pool were filtered through membrane filters (pore size 0.45 μm) at a filtering pressure of one-half atmosphere. The filters were then dried at 60^{o} C for 24 hours, allowed to cool to room temperature in a dessicator, and weighed to the nearest 10^{-4} gram. The organic matter present in the filters was burned in a muffle furnace at 500^{o} C for one hour. Following this, the clays present in the samples were rehydrated (American Public Health Association 1975) and the residue weighed. The major portion of each sample was fixed with acid-Lugol's solution and permanent slides were prepared using a method of concentrating phytoplankton on a membrane filter. Enumeration of the algae on each filter was performed after McNabb (1960) by counting the algae present in 30 random fields on each filter. The taxa observed were usually determined to the specific level, although identification problems infrequently required identification only to genus. Identification of algae was aided by use of two taxonomic references (Tiffany and Britton 1952; Prescott 1964).

Results

Summarized data on temperature, dissolved oxygen, and hydrogen ion concentration collected from May 14, 1979, to July 11, 1979, is presented in Figure 2. Temperature readings displayed are the average of morning and afternoon measurements from mid-depth in pools 3 and 4. The greatest difference in temperature readings between the morning and afternoon of a single day was 2.3° C. Records of temperature in the unshaded pools were not made until late June. Even after this time, however, temperatures never differed more than 1° C between the shaded and unshaded pools on a given date. Mean daily temperature increased from 10.4° C in mid-May to 23.5° C in mid-July.

Dissolved oxygen measurements (mg/liter) are displayed as a function of temperature in percent saturation. Each point represents the mean of six readings (a morning and afternoon reading from each of the 3 streams). Dissolved oxygen readings were obtained at mid-depth in pools 3. The average difference in dissolved oxygen readings between streams was less than 3%. The dissolved oxygen concentration was always somewhat higher during the afternoon. Throughout the period of sampling, the dissolved oxygen concentration remained between 77 and 91 percent saturation.

For each stream, the pH is shown by two lines. One line is pH as recorded at mid-depth in pools 3 (the average between morning and afternoon readings). The other line is the average of readings taken from riffles 3 and 4. Generally, the pH in the riffles was slightly higher than the readings from mid-depth in the pools. There is no clear trend between morning and afternoon readings, although the difference was sometimes greater than 0.5 pH units. The pH in Stream 8 varied between 7.8

and 8.6 with a mean recorded reading of 8.1 for the entire eight-week period. Stream 6 had a mean recorded pH reading of 6.3, varying between 6.0 and 6.6. Stream 5 had a mean of pH 5.3, varying between 5.0 and 5.7. Thus, three clearly defined pH regimes were present within the system throughout the experimental period.

Weekly measurements of four other chemical parameters are presented in Table 1. Specific conductance is similar over time and is always highest in Stream 5 and lowest in Stream 8 due, no doubt, to the sulfuric acid added to the experimental streams. Hardness is similar between the three streams but showed an increase in all three streams between late May and mid-July. As would be expected, the total acidity is by far the greatest in Stream 5 and lowest in Stream 8. Accordingly, alkalinity is lowest in Stream 5; highest in Stream 8.

Since the discharge of the streams was known, it was possible to compute the velocity of flow in riffles and pools, and hence, a theoretical residence time for phytoplankton in the system. Hynes (1970) presents the following equation:

$$V = \frac{D}{wda}$$

where D = discharge, V is stream velocity, w and d are the values for mean stream width and depth, and a is the coefficient representing stream roughness. Rather high coefficients of 4.0 for pools and 2.7 for riffles were computed. These values took into account the dense growth of macrophytes present in the streams. These values were developed using information from Chow (1959). The residence time of stream phytoplankton was computed to be 6-2/3 hours in a pool, and for a riffle it was 1/2 hour

(33 minutes). It follows then that the theoretical exposure time of stream phytoplankton to the various pH regimes is approximately 24-1/2 hours before reaching the sampling area in pools 4.

The overall results of phytoplankton enumerations are shown in Figure 3. It can be seen that the total phytoplankton density differs in each stream. Each stream also differs in its pattern of phytoplankton community development over the course of the sampling period. The density of phytoplankton in Streams 8 and 6 was highest during the first month of sampling. Conversely, the phytoplankton in Stream 5 increased in density gradually, reaching its highest value by the second month of sampling. Phytoplankton density at the end of the sampling period (mid-July) was low in all three streams. Figure 4 displays relative phytoplankton biomass as indicated by *in vivo* chlorophyll fluorescence. The trend for chlorophyll *a* in each stream is roughly equivalent to the trends in total algal density. Figure 5 shows the ash-free dry weights, derived from phytoplankton samples collected on all sampling dates.

Measurements of phytoplankton community structure were calculated from the phytoplankton data and are presented in Table 2. Simpson's Index of Diversity (Simpson 1949) measures the probability that two individuals picked at random from one community will belong to different species. Thus, a value of zero would indicate that there is only one taxon in the community and a value of one indicates that the community is infinitely diverse. By this measurement, it becomes evident that all three streams have a similar diversity over the course of the experimental period. Morisita's Index of Community Similarity (Morisita 1959) is derived from Simpson's index, and measures the probability that two

individuals taken from different communities will belong to the same taxon. In this case, a value of zero would indicate that there is no similarity between the two communities, and a value of one would indicate that the compared communities are identical. During each sampling period, the phytoplankton communities of each stream are compared to each other (Table 2). A clear trend of divergence emerges, each stream community becoming more dissimilar from the others over the course of the study.

Figure 6 provides a detailed look at growth trends for eight dominant algal taxa. These taxa constituted as much as 94% of the algae present and always represented the majority of each stream's phytoplankton community. By July 11 (at the end of the study), these eight taxa were not nearly as important as in earlier sampling periods. They were not, however, replaced by other species becoming dominant.

The algae of Stream 5, the most acidic stream, showed a pattern of development markedly different from the patterns exhibited by Streams 6 and 8. During mid to late June, each of the eight taxa had a higher density in Stream 5 than in the other two streams. But, over the duration of exposure to acid, the density of algae in Stream 5 declined more rapidly than in the other two streams.

The overall species composition was similar in all streams throughout the course of the study. *Cyclotella meneghiniana* was the most dominant single species; two species of *Melosira* together accounted for more biomass than *C. meneghiniana* due to the large size of chains that *Melosira* forms. The other five taxa were always present in the samples. Diatoms (Bacillariophyceae) were overwhelmingly the dominant algal group in the Monticello streams. Diatoms averaged 89.6% of the algae enumerated from phytoplankton

samples (with a range from 81—98%). The Chlorophyta, mainly *Ulothrix*, *Scenedesmus*, and *Spirogyra*, were also common. Very few of the 48 algal taxa identified (Appendix 2) displayed intolerance to any of the three pH regimes. However, *Eunotia lunaris* was never present in the ambient stream, while it became very common in Stream 5. Three species of *Navicula* were present in Streams 8 and 6, but they never were found in Stream 5.

Discussion

The Monticello streams provided an unique replicate system whereby physical and chemical parameters could be controlled in a field situation. The stream beds were uniform and the rate of flow remained constant in all three systems. Water from the Mississippi River, nearly saturated with dissolved oxygen was provided to all streams. The temperature in each stream was nearly identical to the others at all times and water hardness was the same. These streams were ideal habitats for the colonization of algae and macrophytes. The density of algae in the sampled pools developed significant densities in each stream according to the environmental conditions in that stream. Residence time for algae in the streams was undoubtedly longer than calculated for streamwater (24 hours) since most algae probably grew within the artificial streams themselves, after an initial inoculation of that particular taxon to the streams.

The addition of sulfuric acid to the experimental streams altered the pH regimes there, making each of the three streams distinct from the others. The acid addition raised the specific conductance and total acidity of water in the experimental channels; bicarbonate alkalinity

correspondingly declined and was especially low in Stream 5. Thus, the regulation of most parameters and the alteration of pH provided an excellent opportunity for studying the response of stream phytoplankton to the stress of increased hydrogen ion concentration.

Colonization of the streams with different types of algae from the Mississippi River was equally successful in each stream, as measured by the index of diversity. Diversity of algae was quite high in each stream throughout the course of the experimental period. Patrick et al. (1968) in a study of stream diatoms found little or no change in the diatom diversity at pH 5.2 in one experiment. However, many authors report a decrease in the diversity of the phytoplankton community with increasingly acidic conditions (Johnson et al. 1970; Kwaitkowski and Roff 1976; Leivestad et al. 1976; Yan and Stokes 1976; and Yan 1979). The diversity in each stream of the present study was represented by a similar species composition consisting mainly of diatoms.

Although community diversity was similar under these three pH regimes, the similarity between communities decreased over the course of the six week experimental period. This can be considered an effect of varying conditions (mainly pH) between the three streams, since the other variables changed in concert.

Biomass showed similar patterns whether measured by *in vivo* chlorophyll fluorescence or as the density of algal cells. The algae in Streams 6 and 8 attained their maximum biomass during the first month of sampling. There is a lag in the maxima of diatoms in Stream 5, in that the point of highest algal biomass occurs during the beginning of the second month. Patrick et al. (1968) found that attached stream diatoms at pH 5.2 had a

slower division rate than those in more neutral streams. A slower division rate due to less than an ideal pH environment may have caused the lag in the maxima of diatoms in Stream 5. Biomass as measured by dry weights and ash-free dry weights gave inconclusive results. Sometimes, *Spirogyra* and other filamentous benthic algae were included in water samples, and this may have contributed to the variable results.

By mid-July phytoplankton density was at its lowest in all three streams (Figures 3 and 6). A combination of factors, such as reduced nutrient supply or steadily rising water temperature, may have caused the phytoplankton decline to occur. In the Monticello streams, dense macrophyte stands (chiefly *Potamogeton crispus*) had accumulated in all three streams by mid-July. This submerged vegetation led to reduced light penetration in the streams, which could also have reduced phytoplankton growth.

Figure 6 shows that the most dominant algal taxa found in the Monticello streams occur in great abundance under each of the pH conditions. Nygaard (1956) reported that *Cyclotella meneghiniana* is an alkaliphilous species, although it showed no such preference in this study. Round (1964) found *Melosira distans* to be abundant under extremely acidic conditions, and Merilainen (1967) reported it to be acidophilous. In confirmation, it should be noted that this species reached its greatest abundance in Stream 5 in this study. *Melosira italica* (also reaching its greatest abundance in Stream 5) is reported as alkaliphilous (Nygaard 1956; Merilainen 1967). Patrick et al. (1968) found the abundance of *Fragilaria crotonensis* to be reduced at pH 5.2 (compared to more neutral conditions). Nygaard (1956) reported it to be alkaliphilous. No such

preference for alkaline conditions was indicated by this species in Monticello streams. *Nitzschia palea*, which did best in Stream 5, is reported to be indifferent to pH (Merilainen 1967), but has been noted under acid conditions (Round 1964; Patrick et al. 1968).

Just which physiologic features of specific algae actually determine their tolerance to acidic conditions is a rather unstudied area of phycology. It has been suggested that cells which are tolerant of acid environments are able to restrict hydrogen ions from entering their cells (Cassin 1974), while intolerant types may not have this ability. Clymo (1963) found that the acidophilous moss *Sphagnum* has the ability to release H^+ ions into the surrounding medium and exchange these ions for other cations that it takes up. Polyuronic acids in the cell boundary appear to be involved in this exchange. Lane and Burris (1979) suggested that acid-tolerant species either exclude hydrogen ions or have adapted to low internal pH levels. Internal pH determined in *Chlorella pyrenoidosa* showed that internal pH does indeed vary with environmental pH, although the magnitude of variation internally is not as great as the external fluctuation.

In the present study, it is possible that the potential stress of the average pH 5.3 in Stream 5 is ameliorated by otherwise optimum conditions for algal growth. Temperature in the streams was in the range of optimal conditions for the growth of the taxa present there (Patrick 1969). Also, most chemical characteristics of the Monticello streams were similar to those in circumneutral waters and not of naturally occurring acid waters. Even without the contribution of high hydrogen ion concentration in the experimental channels, the conductivity of the water was

rather high. A medium water hardness of similar value existed in all three streams, regardless of the adjusted acidity.

The mechanism of ion transfer in phytoplankton may be altered by low pH levels and these mechanisms may be less altered when levels of salt are at high concentrations, as has been found with other freshwater organisms. Tolerance of trout to low pH is increased when the concentrations of salts is increased (Leivestad et al. 1976). Heavy metal tolerance by algae can also be enhanced by the presence of other salts. Hargreaves and Whitton (1976b) found the tolerance of *Hormidium rivulare* to zinc was increased by the presence of calcium, phosphorus, or magnesium in the growth medium. Patrick et al. (1968) stated that when otherwise circumneutral conditions occurred in a stream, the altering of one parameter (pH) would not necessarily be expected to change diatom community structure significantly. Accessory environmental factors can often act to increase tolerance to a lethal agent (Warren 1971). In the present study, a pH of 5.3 did not alter phytoplankton community diversity or species composition (compared to more neutral conditions). However, the pattern of community development was markedly different between an acid stream and streams of higher pH, suggesting that high acidity may slow the division rate of algal cells there, decreasing productivity. Certainly pH 5.3 proved to be a sublethal value for the diatoms present but further increases in acidity could undoubtedly result in more drastic changes in community structure. The somewhat equivocal results from the various studies cited suggest that further investigation is needed to clarify relationships between the phytoplankton community and acidification.

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Table 1. Summary of four chemical characteristics of Monticello streams obtained from weekly samples over the course of the experiment.

Specific Conductance [μmhos · cm^{-1} (25°C)]

Date	Stream 8	6	5
May 26	244	282	293
June 2	281	292	316
June 9	307	312	333
June 16	258	269	276
June 23	293	302	336
June 30	268	275	307
July 7	284	308	340
July 14	291	300	317

Total Acidity (mg/liter)

Date	Stream 8	6	5
May 26	4	43	67
June 2	4	50	92
June 9	4	62	180
June 16	4	65	102
June 23	3	63	113
June 30	5	62	104
July 7	4	64	110
July 14	2	62	116

Bicarbonate Alkalinity (mg $CaCo_3$/liter)

Date	Stream 8	6	5
May 26	119	46	10
June 2	130	56	8
June 9	144	55	10
June 16	134	60	17
June 23	158	57	6
June 30	146	66	9
July 7	152	62	7
July 14	148	69	8

Hardness (mg/liter)

Date	Stream 8	6	5
May 26	119	121	121
June 2	130	132	133
June 9	144	144	144
June 16	134	134	136
June 23	158	154	154
June 30	146	150	152
July 7	152	150	150
July 14	148	152	152

Table 2. Measurements of phytoplankton community structure in all streams on each sampling date.

Stream	No. Taxa Observed	Simpson's Index of Diversity	Morisita's Index of Community Similarity			
		May 30				
8	25	0.08			Stream	
6	25	0.81			6	5
5	21	0.66	Stream	8	0.33	0.51
				6	—	0.33
		June 13				
8	27	0.80			Stream	
6	29	0.84			6	5
5	27	0.80	Stream	8	0.21	0.24
				6	—	0.29
		June 27				
8	27	0.80			Stream	
6	29	0.84	Stream	8	6	5
5	28	0.86		6	0.20	0.19
					—	0.17
		July 11				
8	32	0.89			Stream	
6	22	0.77			6	5
5	26	0.89	Stream	8	0.18	0.11
				6	—	0.17

Figure 1. The three Monticello artificial streams. Arrow indicates the direction of flow. Dark patching indicates upstream area shaded by canvas. mc = mixing compartment, 1—4 = pools one through four, b—d = riffles two through four.

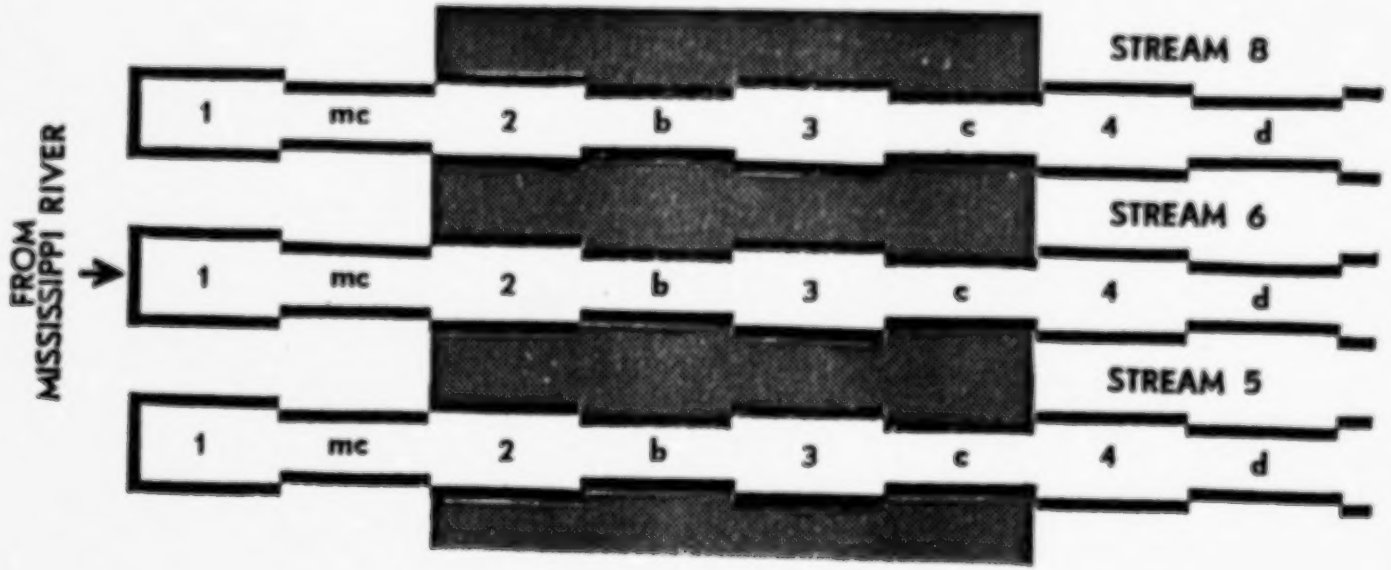

FROM
MISSISSIPPI RIVER
STREAM 8
STREAM 6
STREAM 5
1
mc
2
b
3
c
4
d

Figure 2. (a) Average mid-depth temperature in shaded and unshaded pools over the course of the experiment.

▰▰ = shaded pool temperature, ▬▬ = unshaded pool temperature.

(b) Average percent saturation of dissolved oxygen at mid-depth in pools over the course of the experiment.

(c) Average hydrogen ion concentration for each stream over the course of the experiment. ——— = pH in riffles, ▪▪▪▪▪▪▪▪ = pH in pools, ● = phytoplankton sampling dates.

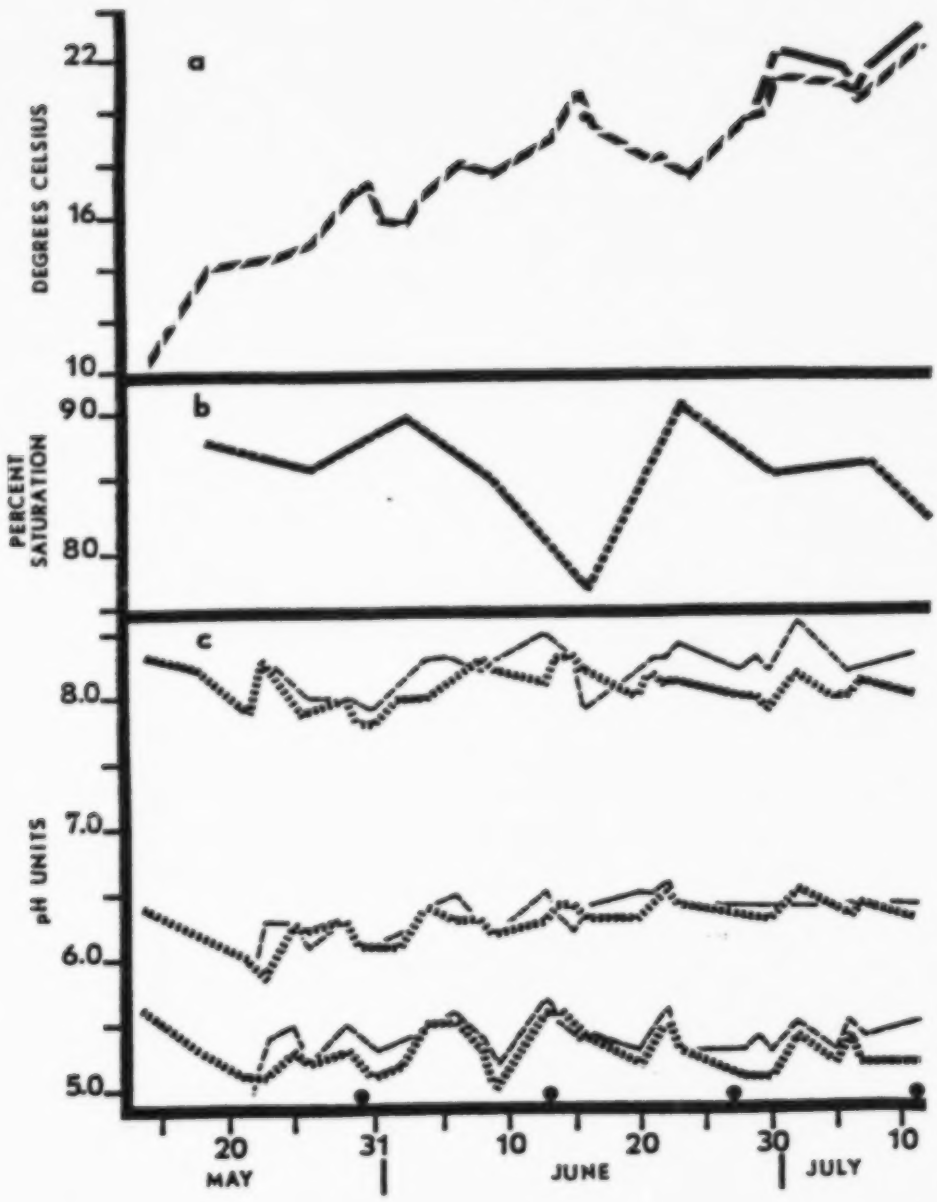

a
22
16
10
DEGREES CELSIUS
b
90
80
PERCENT SATURATION
c
8.0
7.0
6.0
5.0
pH UNITS
20
MAY
31
10
JUNE
20
30
JULY
10

Figure 3. Overall phytoplankton density in all streams on each sampling date. ▬ ▬ = Stream 8, ▪▪▪▪▪▪▪ = Stream 6, ◤◢◤◢ = Stream 5.

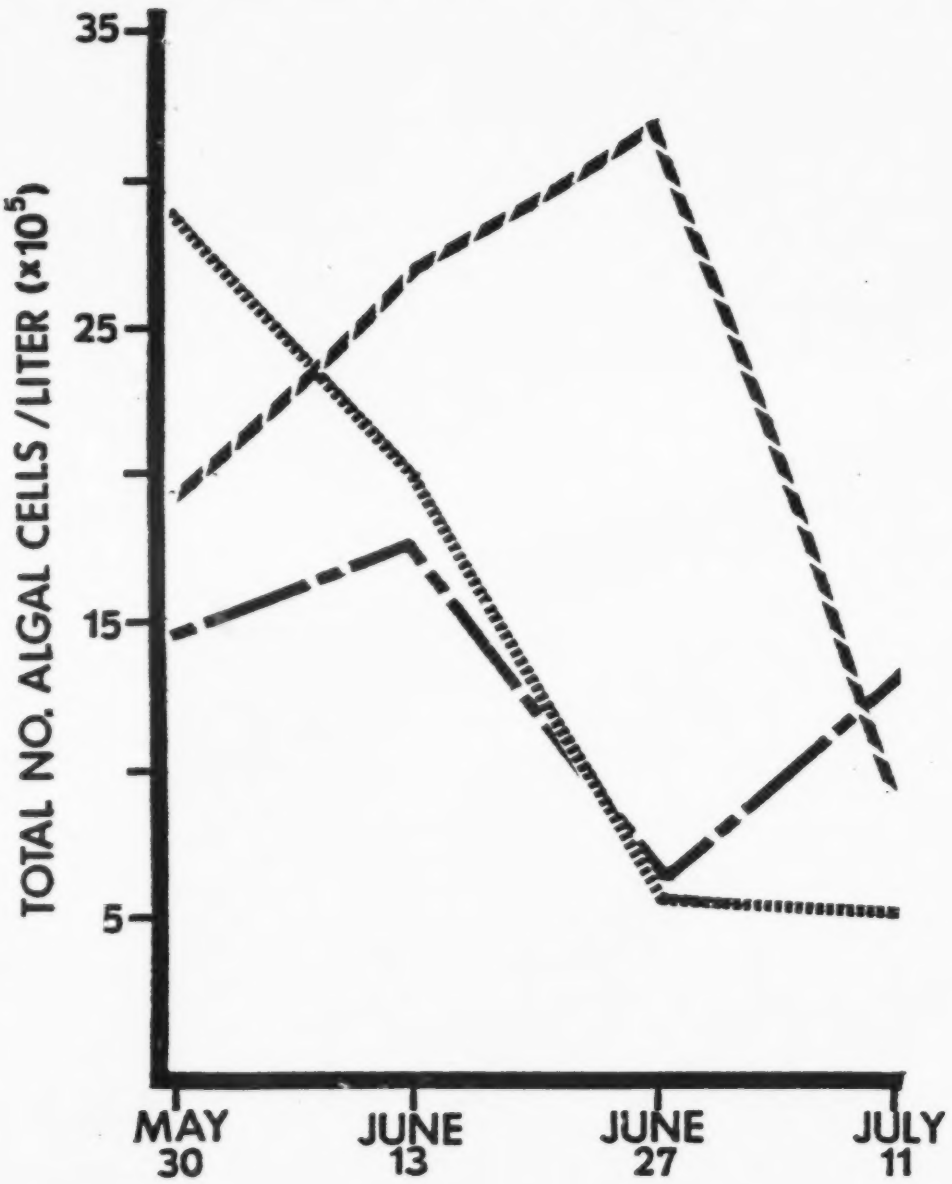

TOTAL NO. ALGAL CELLS /LITER (×10⁵)
35
25
15
5
MAY 30
JUNE 13
JUNE 27
JULY 11

Figure 4. *In vivo* Chlorophyll fluorescence of phytoplankton in all streams on each sampling date. ▬▬ = Stream 8, ▬ ▬ = Stream 6, ▬ ▬ ▬ = Stream 5.

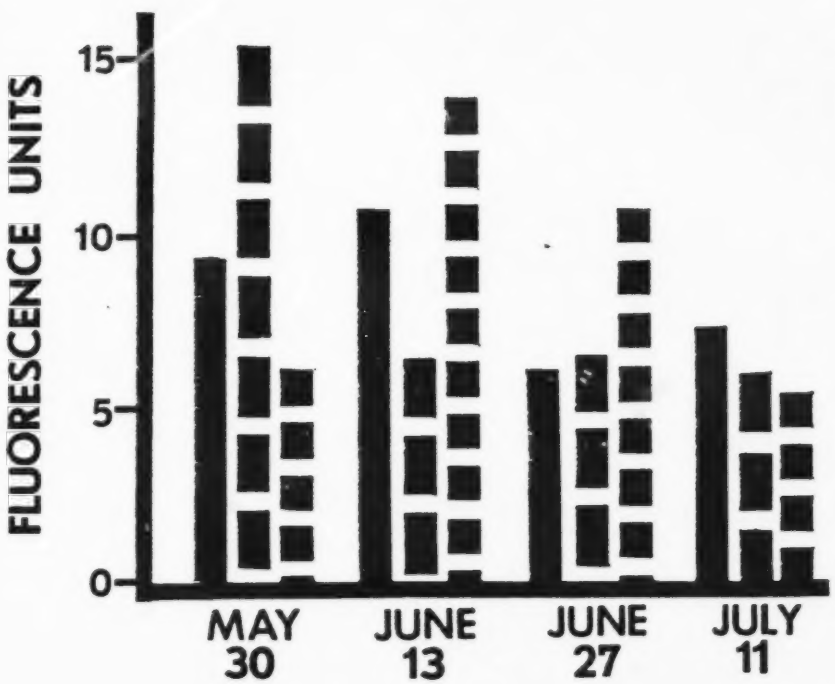

FLUORESCENCE UNITS
15
10
5
0
MAY 30
JUNE 13
JUNE 27
JULY 11

Figure 5. Ash-free dry weight of phytoplankton samples from all streams on each sampling date. Each bar represents the average weight of two duplicate samples. ▬▬ = Stream 8, ▬▪ = Stream 6, ▬▪▪ = Stream 5.

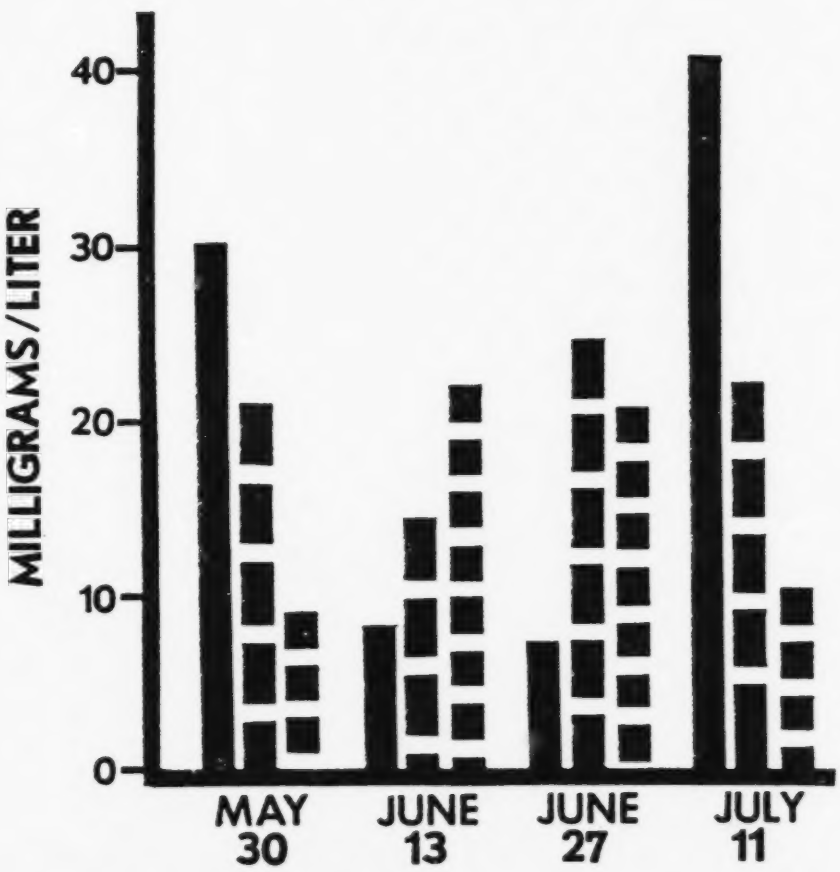
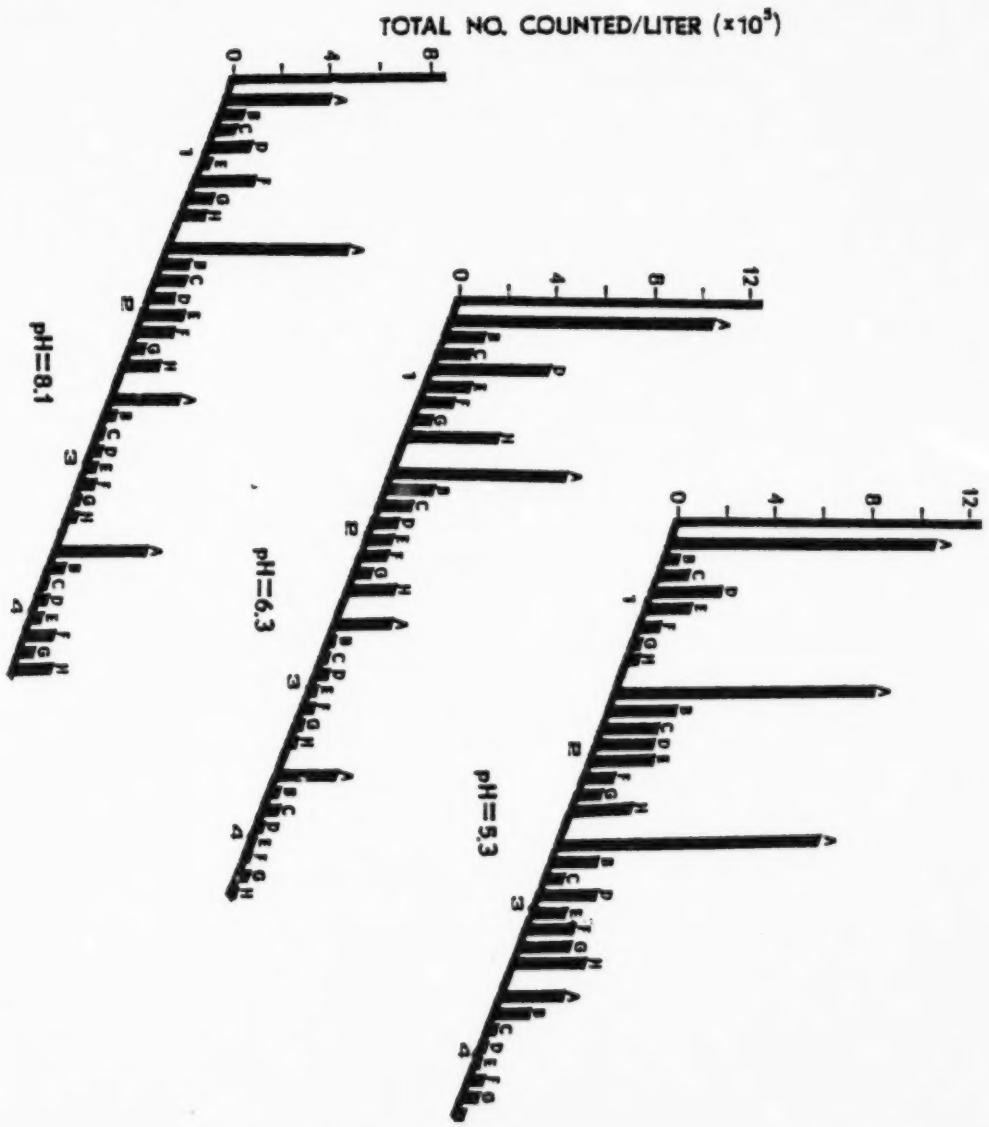

MILLIGRAMS/LITER
40
30
20
10
0
MAY 30
JUNE 13
JUNE 27
JULY 11

Figure 6. Density of eight major phytoplankton taxa in three distinct pH regimes on each sampling date. 1—4 = the four sampling dates (May 30, June 13, June 27, July 11 respectively), A = *Cyclotella meneghiniana*, B = *Melosira distans*, C = *Melosira italica*, D = *Asterionella formosa*, E = *Fragilaria crotonensis*, F = *Navicula* spp. (seven species), G = *Nitzschia* spp. (three species), H = Total Chlorophyta taxa


TOTAL NO. COUNTED/LITER (×10⁵)
0
4
8
12
pH=8.1
pH=6.3
pH=5.3
1
2
3
4

Appendix 1

Metal Characteristics of the Sulfuric Acid

Metal Content	Maximum Amount (mg/liter)
Iron	35
Arsenic	3
Manganese	0.3
Zinc	0.4
Selinum	1.2
Copper	1
Nickel	0.3
Lead	4
Sulfur Dioxide	20
Chloride	2
Nitrate	3
Fixed Residue	75

Brand:

ASARCO H_2SO_4, Commercial 66^{o}

Bought From:

Thompson-Hayward Chemical Company, Minneapolis, Minnesota.

Appendix 2

Phytoplankton Observed in Experimental Streams

Taxa	Stream		
	8	6	5
Chrysophyta			
Asterionella formosa	VC[1]	VC	VC
A. gracillima	VR	VR	—
Cocconeis placentula	R	C	VC
Cyclotella bodanica	R	R	R
C. meneghiniana	VC	VC	VC
Cymbella cistula	R	R	R
C. gracilis	R	R	R
Diatoma vulgare	VC	VC	VC
Epithemia turgida	VR	—	—
Eunotia lunaris	VR	R	VC
Fragilaria capucina	R	R	C
F. crotonensis	VC	VC	VC
Gomphonema constrictum	—	VR	—
G. montanum	—	R	—
G. olivaceum	—	—	VR
Gyrosigma acuminatum	R	—	—
G. scaproides	VR	VR	VR
Melosira distans	VC	VC	VC
M. italica	VC	VC	VC
Meridion circulare	VC	R	C
Navicula cryptocephala	C	C	VR
N. cuspidata	R	—	—
N. platystoma	R	—	—
N. radiosa	VR	VR	—
N. tuscula	C	R	R
N. viridula	R	R	R
N. spp.	C	R	C
Nitzschia linearis	R	R	VC
N. palea	R	R	VC
Nitzschia sigmoides	VR	VR	VR
Rhoicosphenia curvata	R	R	R
Stephanodiscus niagare	R	R	R
Synedra ulna	R	VC	R
Tabellaria fenestrata	R	—	R

Taxa	Stream		
	8	6	5
Chlorophyta			
Ankistrodesmus sp.	VR	—	—
Closterium sp.	—	—	VR
Mougeotia sp.	C	C	C
Pediastrum angulusom	—	R	—
P. duplex	R	VR	R
Scenedesmus obliquus	C	C	C
Spi·ogyra sp.	C	C	C
Ulothrix zonata	C	VC	R
Unidentified colonial flagellate	—	C	—
Unidentified unicell. flagellate	R	R	VR
Cyanophyta			
Anabaena sp.	R	R	R
Anacystis marginata	—	—	VR
Aphanizomenon sp.	VR	R	—
Synechocystis aquatis	R	C	C

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1. VC = very common (found at a density over 10^4 cells/liter at least once)

   C = common (between 50,000—10^4 cells/liter at least once)

   R = rare (always under 50,000 cells/liter)

   VR = very rare (found only once)